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FILE 'CAPLUS' ENTERED AT 09:43:22 ON 02 SEP 2005

=> S HYDROLASE;S ESTERASE;S LIPASE;S PROTEASE;S STEREO?;S REGIO?
         20099 HYDROLASE
          8330 HYDROLASES
L1       24371 HYDROLASE
                (HYDROLASE OR HYDROLASES)


         30476 ESTERASE
         11018 ESTERASES
L2       34996 ESTERASE
                (ESTERASE OR ESTERASES)


         44902 LIPASE
          8133 LIPASES
L3       46155 LIPASE
                (LIPASE OR LIPASES)


         88966 PROTEASE
         33090 PROTEASES
L4      104029 PROTEASE
                (PROTEASE OR PROTEASES)


L5      235071 STEREO?


L6     1298988 REGIO?

=> S SPECTROPHOTOM?;S SPECTRAL PHOTOMET?
L7      170512 SPECTROPHOTOM?


        356110 SPECTRAL
             5 SPECTRALS
        356112 SPECTRAL
                (SPECTRAL OR SPECTRALS)
         71585 PHOTOMET?
L8         332 SPECTRAL PHOTOMET?
                (SPECTRAL(W)PHOTOMET?)

=> S (L1,L2,L3,L4) AND (L5,L6)
L9       19265 ((L1 OR L2 OR L3 OR L4)) AND ((L5 OR L6))

=> S L9 AND (L7,L8)
L10         44 L9 AND ((L7 OR L8))

=> D 1-44 CBIB ABS

L10  ANSWER 1 OF 44  CAPLUS  COPYRIGHT 2005 ACS on STN
2005:401016   Document No. 143:72947  Proteomic analysis of the proteins
     expressed by hydrogen peroxide treated cultured human dermal microvascular
     endothelial cells.  Ha, Moon Kyung; Chung, Kee Yang; Bang, Dongsik; Park,
     Yoon Kee; Lee, Kwang Hoon (Department of Dermatology and Cutaneous Biology
     Research Institute, Seoul, S. Korea).  Proteomics, 5(6), 1507-1519
     (English) 2005.  CODEN: PROTC7.  ISSN: 1615-9853.  Publisher: Wiley-VCH
     Verlag GmbH & Co. KGaA.
AB   Reactive oxygen species (ROS) have been traditionally regarded as toxic
     byproducts of aerobic metabolism  However, ROS also act as intracellular
     signaling mols. and can mediate phenotypes in vascular endothelial cells, which
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may be physiol. or pathol. in nature. To clarify the mol. mechanisms of ROS signaling, the authors examined hydrogen peroxide (H2O2)-responsive proteins in cultured human dermal microvascular endothelial cells (HMVEC) using proteomic tools. Protein expression in HMVEC was studied after they had been exposed to low- and high-levels of H2O2 for various times, and intracellular ROS production was examined by flow cytometer and UV spectrophotometer. Proteins obtained from dose- and time-dependent series were separated by two-dimensional gel electrophoresis and tentatively identified by matrix-assisted laser desorption-time of flight mass spectrometry, by matching the tryptic mass maps obtained with entries in the NCBI and Swiss-Prot protein sequence database. At least 163 proteins were changed by H2O2, and 60 proteins were identified. Oxidative stress triggered dramatic change in the expression of proteins in primary microvessel endothelial cells, and their mapping to cellular process provided a view of the ubiquitous cellular changes elicited by H2O2. These results could provide a framework for the understanding of the mechanisms of cellular redox homeostasis and H2O2 metabolism in microendothelium environment in various biol. processes as well as pathol. conditions.

L10 ANSWER 2 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN
2004:602491 Document No. 142:172047 Directed Evolution of Epoxide Hydrolase from A. radiobacter toward Higher Enantioselectivity by Error-Prone PCR and DNA Shuffling. van Loo, Bert; Spelberg, Jeffrey H. Lutje; Kingma, Jaap; Sonke, Theo; Wubbolts, Marcel G.; Janssen, Dick B. (Biochemical Laboratory, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Groningen, 9747 AG, Neth.). Chemistry & Biology, 11(7), 981-990 (English) 2004. CODEN: CBOLE2. ISSN: 1074-5521. Publisher: Cell Press.

AB The enantioselectivity of epoxide hydrolase from Agrobacterium radiobacter (EchA) was improved using error-prone PCR and DNA shuffling. An agar plate assay was used to screen the mutant libraries for activity. Screening for improved enantioselectivity was subsequently done by spectrophotometric progress curve anal. of the conversion of para-nitrophenyl glycidyl ether (pNPGE). Kinetic resolns. showed that eight mutants were obtained with up to 13-fold improved enantioselectivity toward pNPGE and at least three other epoxides. The large enhancements in enantioselectivity toward epichlorohydrin and 1,2-epoxyhexane indicated that pNPGE acts as an epoxyalkane mimic. Active site mutations were found in all shuffled mutants, which can be explained by an interaction of the affected amino acid with the epoxide oxygen or the hydrophobic moiety of the substrate. Several mutations in the shuffled mutants had additive effects.

L10 ANSWER 3 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN
2003:202925 Document No. 138:236935 Protein sequences of human antigen HE4a and diagnosis of carcinomas. Schummer, Michel; Hellstrom, Ingegerd; Hellstrom, Karl Erik; Ledbetter, Jeffrey A.; Hayden-ledbetter, Martha (Pacific Northwest Research Institute, USA). PCT Int. Appl. WO 2003021273 A2 20030313, 85 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-EP9653 20020829. PRIORITY: US 2001-2001/PV316537 20010829.

AB The invention is directed to compns. and methods for the detection of a malignant condition, and relates to the discovery of soluble and cell surface forms of HE4a polypeptides, including HE4a that is overexpressed in ovarian carcinomas. In particular the invention provides a nucleic acid sequence encoding HE4a, and also provides a method of screening for the presence of a malignant condition in a subject by detecting reactivity of an antibody specific for a HE4a polypeptide with a mol. naturally occurring in soluble and/or cell surface form in a sample

from such a subject, and by hybridization screening using an HE4a nucleotide sequence, as well as other related advantages.

L10 ANSWER 4 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN
2002:882745 Document No. 138:233775 A selective HIV-protease assay based on a chromogenic amino acid. Badalassi, Fabrizio; Nguyen, Hong Khan; Crotti, Paolo; Reymond, Jean-Louis (Departement fur Chemie und Biochemie, Universitat Bern, Bern, CH-3012, Switz.). Helvetica Chimica Acta, 85(10), 3090-3098 (English) 2002. CODEN: HCACAV. ISSN: 0018-019X. OTHER SOURCES: CASREACT 138:233775. Publisher: Verlag Helvetica Chimica Acta.

AB (2S,3S)-2-Amino-3-hydroxy-5-(4-nitrophenoxy)pentanoic acid (5) was prepared stereoselectively as the N-Fmoc, O-(tert-butyl)-protected derivative 5a in eleven steps from Et (E)-4-benzyloxypent-2-enoate (6). This protected amino acid was used for the solid-phase peptide synthesis of oligopeptides, which serve as sequence-specific chromogenic protease substrates when used in the presence of NaIO4 and bovine serum albumin. The peptide 1 (KRAVNle-5-EANleNH2 (Nle = norleucine)) allows detection of HIV-protease activity spectrophotometrically at 405 nm.

L10 ANSWER 5 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN
2002:786505 Document No. 139:193334 Dinitrophenol derivatization of proteolytic products and its application in the assay of protease (s) activity. Bhaskar, Kiran; Pavankumar Shetty, A.; Shareef, Momin M.; Ramamohan, Y.; Taranath Shetty, K. (Department of Neurochemistry, National Institute of Mental Health and Neurosciences, Bangalore, 560 029, India). Journal of Neuroscience Methods, 120(2), 155-161 (English) 2002. CODEN: JNMEDT. ISSN: 0165-0270. Publisher: Elsevier Science B.V..

AB A spectrophotometric method based on dinitrophenol (DNP) derivatization of proteolytic products was developed for monitoring the increase in NH2-groups as a function of protease activity. DNP derivatization of amino acids and proteolytic products was carried out at an alkaline pH of 8.8, in presence of 2,4-dinitrofluorobenzene (DNFB), followed by the stabilization of products by adjusting the pH to .apprx.2.5. Using casein as substrate, under the defined assay conditions for proteases, trichloroacetic acid soluble proteolytic products were derivatized with DNFB reagent. Though alkaline pH favored the DNP derivatization of primary amino compds., the products formed were found to be unstable. However, upon adjusting the pH to 2.5±0.1, DNP derivs. of amino acids and proteolytic products were found to be stable with identical λmax of 395 nm. The utility of the method was evaluated by assaying the proteolytic activities of trypsin and calcium activated neutral protease (CANP). Proteolytic activity was quantified by employing the molar extinction coefficient of DNP derivs. of an equimolar concentration of glutamate and glycine. By employing this method, CANP activity in different regions of rat brain was determined The proposed method to monitor the increase in NH2-end groups as a function of proteolytic activity could be employed to assay the activity of proteases.

L10 ANSWER 6 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN
2001:561418 Document No. 135:239714 Expression, activity, and subcellular localization of testicular hormone-sensitive lipase during postnatal development in the guinea pig. Kabbaj, Ouafae; Holm, Cecilia; Vitale, Maria L.; Pelletier, R.-Marc (Departement de Pathologie et Biologie Cellulaire, Faculte de Medecine, Universite de Montreal, Montreal, QC, H3T 1J4, Can.). Biology of Reproduction, 65(2), 601-612 (English) 2001. CODEN: BIREBV. ISSN: 0006-3363. Publisher: Society for the Study of Reproduction.

AB The present work reports on testicular hormone-sensitive lipase (HSL), the biol. significance of which has been documented in male fertility. The HSL protein levels and enzymic activity were measured, resp., by densitometry of immunoreactive bands in Western blots, performed with antibodies against

recombinant rat HSL, and by spectrophotometry in seminiferous tubules (STf) and interstitial tissue (ITf) enriched fractions generated from neonatal, pubertal, and adult guinea pig testes. In addition, HSL was studied in subcellular fractions obtained from STf isolated from adult testes and in epididymal spermatozoa (Spz). A 104-kDa HSL protein was detected in STf and ITf, the expression and activity of which increased with testicular development. Three immunoreactive bands of 104, 110, and 120 kDa were detected in the lysosomal subfraction, and two bands of 104 and 120 kDa were detected in Spz. The HSL activity was pos. correlated with free (FC) and esterified (EC) cholesterol ratios in STf and ITf, but not with triglyceride (TG) levels, during testicular development. Immunolabeling localized HSL to elongated spermatids and Sertoli cells, where its distribution was stage-dependent, and within the cells lining the excurrent ducts of the testis. The findings of the 104- and 120-kDa HSL immunoreactive bands and of HSL activity in Spz as well, as the detection of the 104-, 110-, and 120-kDa immunoreactive bands in lysosomes, suggest that part of HSL may originate from germ cells and be imported in Sertoli cells. The HSL protein levels and enzymic activity in ITf and STf were pos. correlated with serum testosterone levels during development. To the best of our knowledge, this study is the first to contribute insights regarding the impact of HSL on FC:EC cholesterol ratios and TG levels in the interstitial tissue and tubules in relation to serum testosterone levels during postnatal development, and regarding the immunolocalization of the enzyme in regions of the male gamete consistent with spermatozoa-oocyte interaction.

L10 ANSWER 7 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN

2001:55142 Document No. 134:365735 Directed Evolution of Toluene Dioxygenase from Pseudomonas putida for Improved Selectivity Toward cis-Indandiol during Indene Bioconversion. Zhang, Ningyan; Stewart, Bruce G.; Moore, Jeffrey C.; Greasham, Randolph L.; Robinson, David K.; Buckland, Barry C.; Lee, Chanyong (Department of Bioprocess R&D, Merck Research Laboratories, Rahway, NJ, 07065, USA). Metabolic Engineering, 2(4), 339-348 (English) 2000. CODEN: MEENFM. ISSN: 1096-7176. Publisher: Academic Press.

AB Toluene dioxygenase (TDO) from Pseudomonas putida F1 converts indene to a mixture of cis-indandiol (racemic), 1-indenol, and 1-indanone. The desired product, cis-(1S, 2R)-indandiol, is a potential key intermediate in the chemical synthesis of indinavir sulfate (Crixivan), Merck's HIV-1 protease inhibitor for the treatment of AIDS. To reduce the undesirable byproducts 1-indenol and 1-indanone formed during indene bioconversion, the recombinant TDO expressed in Escherichia coli was evolved by directed evolution using the error-prone polymerase chain reaction (epPCR) method. High-throughput fluorometric and spectrophotometric assays were developed for rapid screening of the mutant libraries in a 96-well format. Mutants with reduced 1-indenol byproduct formation were identified, and the individual indene bioconversion product profiles of the selected mutants were confirmed by HPLC. Changes in the amino acid sequence of the mutant enzymes were identified by analyzing the nucleotide sequence of the genes. A mutant with the most desirable product profile from each library, defined as the most reduced 1-indenol concentration and with the highest cis-(1S, 2R)-indandiol enantiomeric excess, was used to perform each subsequent round of mutagenesis. After three rounds of mutagenesis and screening, mutant 1C4-3G was identified to have a threefold reduction in 1-indenol formation over the wild type (20% vs 60% of total products) and a 40% increase of product (cis-indandiol) yield. (c) 2000 Academic Press.

L10 ANSWER 8 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN

2000:439802 Document No. 133:146455 Trypsin/acrosin inhibitor activity of rat and guinea pig caltrin proteins. Structural and functional studies. Winnica, Daniel E.; Novella, Maria L.; Dematteis, Andrea; Coronel, Carlos E. (Catedras de Quimica Biologica, Facultades de Ciencias Medicas and Ciencias Exactas, Fisicas y Naturales, Universidad Nacional de Cordoba, Cordoba, Argent.). Biology of Reproduction, 63(1), 42-48 (English) 2000. CODEN: BIREBV. ISSN: 0006-3363. Publisher: Society for the Study of

Reproduction.

AB Dramatic inhibition of trypsin activity by rat caltrin and guinea pig caltrin I was spectrophotometrically demonstrated using the artificial substrate benzoylarginyl Et ester. Approx. 6% and 21% of residual proteolytic activity was recorded after preincubating the enzyme with 0.22 and 0.27 μM rat caltrin and guinea pig caltrin I, resp. Reduction and carboxymethylation of the cysteine residues abolished the inhibitor activity of both caltrin proteins. Rat caltrin and guinea pig caltrin I show structural homol. with secretory trypsin/acrosin inhibitor proteins isolated from boar and human seminal plasma and mouse seminal vesicle secretion and share a fragment of 13 amino acids of almost identical sequence (DPVCGTDGH/K/ITYG/AN), which is also present in the structure of Kazal-type trypsin inhibitor proteins from different mammalian tissues. Bovine, mouse, and guinea pig caltrin II, three caltrin proteins that have no structural homol. with rat caltrin or guinea pig caltrin I, lack trypsin inhibitor activity. Rat caltrin, guinea pig caltrin I, and the mouse seminal vesicle trypsin inhibitor protein P12, which also inhibits Ca2+ uptake into epididymal spermatozoa (mouse caltrin I), bound specifically to the sperm head, on the acrosomal region , as detected by indirect immunofluorescence. They also inhibited the acrosin activity in the gelatin film assay. Caltrin I may play an important role in the control of sperm functions such as Ca2+ influx in the acrosome reaction and activation of acrosin and other serine- proteases at the proper site and proper time to ensure successful fertilization.

L10 ANSWER 9 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN

1999:532908 Document No. 131:283124 Characterization of a human MHC class III region gene product with S-thioesterase activity. Aguado, Begona; Campbell, R. Duncan (MRC Immunochemistry Unit, Department of Biochemistry, Oxford University, Oxford, OX1 3QU, UK). Biochemical Journal, 341(3), 679-689 (English) 1999. CODEN: BIJOAK. ISSN: 0264-6021. Publisher: Portland Press Ltd..

AB Palmitoylated proteins contain a 16-carbon saturated fatty acyl group that is post-translationally attached by a labile thioester bond. These modified proteins are mainly membrane-bound; the lability of the thioester bond allows the process to be reversible, a unique property of this modification. We report here that the gene for G14, located in the class III region of the human MHC, encodes a polypeptide with significant sequence similarity to mammalian palmitoyl protein thioesterase (PPT1), an enzyme that removes palmitate from palmitoylated proteins. The gene for G14, also known as PPT2, is transcribed as at least five different transcripts, which are expressed in different cell lines of the immune system. Immunopptn. of these mammalian cells, with an anti-G14 antiserum, showed a specific band of approx. 42 kDa in cell exts. and supernatants. Expression of the G14 cDNA in the baculovirus system revealed that it encoded a secreted glycosylated polypeptide with S-thioesterase activity. The enzymic activity of the recombinant G14 protein was further characterized in quant. spectrophotometric assays, which revealed that it had the highest S-thioesterase activity for the acyl groups palmitic and myristic acid followed by other long-chain acyl substrates. The S-thioesterase activity of the G14 protein was found to be considerably higher in supernatants than in cell exts., which was consistent with the protein's being secreted. The G14 polypeptide contains, in addition to an N-terminal lipase domain, a C-terminal domain common to the cytokine receptor superfamily, which might determine the substrate specificity and/or the protein target of the G14 protein.

L10 ANSWER 10 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN

1999:328341 Document No. 131:165987 Cloning of Prevotella intermedia loci demonstrating multiple hemolytic domains. Beem, J. E.; Nesbitt, W. E.; Leung, K.-P. (Department of Oral Biology and Periodontal Disease Research Center, College of Dentistry, University of Florida, Gainesville, FL, 32610, USA). Oral Microbiology and Immunology, 14(3), 143-152 (English) 1999. CODEN: OMIMEE. ISSN: 0902-0055. Publisher: Munksgaard International Publishers Ltd..

AB A gene bank was created from Prevotella intermedia strain 27 chromosomal DNA, and a clone was isolated that conferred the expression of two sep. modes of hemolytic activity in recombinant Escherichia coli. The original recombinant hemolytic strain (EB34) contained plasmid, pEB34, with a 5.6-kb insert from Sau3AI-digested P. intermedia strain 27 chromosomal DNA cloned into the BamHI site of pUC18. EB34 and deletion subclones were tested for expression of hemolytic activity in a standard tube assay, measuring lysis of erythrocytes spectrophotometrically as a function of Hb release. Cell suspensions of EB34 demonstrated a dose-dependent hemolytic activity, inhibitable by proteases, and heat treatment but not dependent on calcium ions, and not inhibitable by osmoprotectants. Cell-free lysates also demonstrated a heat inhibitable, dose dependent hemolytic activity. Sub-cloning expts. localized the hemolytic region of the insert to a 3.9-kb fragment under direction of the lac promoter. Sequence anal. of the entire insert revealed the presence of multiple open reading frames (1 to 3) in this region which correlated to different forms of hemolytic expression, such that subclones containing all open reading frames 1 to 3 demonstrated strong hemolytic phenotype on blood plates and in the tube assay. Subclones containing only ORF1 demonstrated hemolysis on plates, but not in the tube assay. Subclones containing only open reading frames 2 and 3, but not ORF1 demonstrated hemolysis in the tube assay but not on plates. Homol. searches of DNA and protein databases have not revealed significant homologies with reported hemolysins or proteins in any of the open reading frames.

L10 ANSWER 11 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN
1999:315432 Document No. 131:99244 Rat liver serine dehydratase. Bacterial expression and two folding domains as revealed by limited proteolysis. Ogawa, Hirofumi; Takusagawa, Fusao; Wakaki, Kunihiko; Kishi, Hiroyuki; Eskandarian, Mohammad R.; Kobayashi, Masashi; Date, Takayasu; Huh, Num-Ho; Pitot, Henry C. (Department of Biochemistry, Faculty of Medicine, Toyama Medical and Pharmaceutical University, Toyama, 930-0194, Japan). Journal of Biological Chemistry, 274(18), 12855-12860 (English) 1999. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB A pCW vector harboring rat liver serine dehydratase (I) cDNA was expressed in Escherichia coli. The expressed level was .apprx.5-fold higher in E. coli BL21 than in E. coli JM109 cell extract; the former lacked 2 kinds of proteases. Immunoblot anal. revealed the occurrence of a derivative other than I in the JM109 cell extract Recombinant I was purified to homogeneity. Staphylococcus aureus V8 protease and trypsin cleaved I at Glu-206 and Lys-220, resp., with concomitant loss of enzyme activity. Spectrophotometrically, the nicked enzyme showed a .apprx.50% reduced capacity for binding of the coenzyme, pyridoxal phosphate, and no spectral change in the CD spectrum in the region at 300-480 nm, whereas CD spectra of both enzymes in the far-UV region were similar, suggesting that proteolysis impairs coenzyme binding without an accompanying gross change of the secondary structure. Whereas the nicked enzyme behaved like the intact enzyme on Sephadex G-75 column chromatog., it was dissociated into 2 fragments on the column containing 6M urea. Upon removal of urea, both fragments spontaneously refolded. These results suggest that I consists of 2 folding domains connected by a region that is very susceptible to proteases.

L10 ANSWER 12 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN
1999:266594 Document No. 131:99076 A Continuous Spectrophotometric Assay for P450 BM-3, a Fatty Acid Hydroxylating Enzyme, and Its Mutant F87A. Schwaneberg, Ulrich; Schmidt-Dannert, Claudia; Schmitt, Jutta; Schmid, Rolf Dieter (Institut fur Technische Biochemie, Universitat Stuttgart, Stuttgart, 70569, Germany). Analytical Biochemistry, 269(2), 359-366 (English) 1999. CODEN: ANBCA2. ISSN: 0003-2697. Publisher: Academic Press.

AB Cytochrome P 450 BM-3 from Bacillus megaterium catalyzes the subterminal hydroxylation of medium- and long-chain fatty acids at the positions ω-1, ω-2, and ω-3. A rapid and continuous spectrophotometric activity assay for

cytochrome P 450 BM-3 based on the conversion of p-nitrophenoxycarboxylic acids (pNCA) to ω-oxycarboxylic acids and the chromophore p-nitrophenolate was developed. In contrast to the commonly used activity assays for this enzyme, relying on the consumption of oxygen or NADPH or the use of 14C-labeled carboxylic acids, the pNCA assay can even be used with crude exts. of the recombinant enzyme from lysed Escherichia coli cells. The kinetics of p-nitrophenolate formation are directly measured at a wavelength of 410 nm using a spectrophotometer or microtiter plate reader. Sensitivity of the assay is greatly enhanced if p-nitrophenoxydodecanoic or p-nitrophenoxypentadecanoic acid are used with the F87A mutant instead of the wild-type P 450 BM-3 enzyme. (c) 1999 Academic Press.

L10 ANSWER 13 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN
1997:752604 Document No. 128:84325 Stereoselective inhibition of human butyrylcholinesterase by phosphonothiolate analogs of (+)- and (-)-cocaine. Berkman, Clifford E.; Underiner, Gail E.; Cashman, John R. (SEATTLE BIOMEDICAL RESEARCH INSTITUTE, SEATTLE, WA, 98109, USA). Biochemical Pharmacology, 54(11), 1261-1266 (English) 1997. CODEN: BCPCA6. ISSN: 0006-2952. Publisher: Elsevier Science Inc..

AB The hydrolysis of cocaine (benzoylecgonine Me ester) to ecgonine Me ester by human butyrylcholinesterase (BuChE; EC 3.1.1.8) has been shown previously to constitute an important means to detoxicate this material to pharmacol. inactive metabolites. The naturally occurring (-)-cocaine is hydrolyzed to ecgonine Me ester approx. 2000 times slower than the unnatural (+)-cocaine isomer. In good agreement with previous studies, (-)-cocaine bound to human BuChE with relatively good affinity and competitively inhibited the hydrolysis of the spectrophotometric substrate butyrylthiocholine with a Ki value of 8.0 μM. Similarly, (+)-cocaine also showed relatively high affinity for the human BuChE and competitively inhibited butyrylthiocholine hydrolysis with a Ki value of 5.4 μM. The phosphonothiolates corresponding to the transition state analogs for both (-)- and (+)-cocaine hydrolysis were synthesized and tested as inhibitors of human BuChE-catalyzed hydrolysis of butyrylthiocholine. The phosphonothiolate corresponding to the transition state for (-)-cocaine hydrolysis was a competitive inhibitor with a Ki value of 55.8 μM. The phosphonothiolate corresponding to the transition state for (+)-cocaine hydrolysis gave a Ki value of 25.9 μM, but, in addition, it also showed irreversible inhibition with a ki of inactivation of 68.8 min-1 M-1. It is likely that the mechanism-based inhibitor described herein may find use as a mechanistic probe of butyrylcholinesterase action and also possibly aid in the purification of this class of esterases.

L10 ANSWER 14 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN
1997:641923 Document No. 127:328194 Spectrophotometric determination of the positional specificity of nonspecific and 1,3-specific lipases. Farias, Ricardo N.; Torres, Merce; Canela, Ramon (Dep. Bioquim. Nutricion, Inst. Superior Invest. Biol. (CONICET-UNT), Inst. Quim. biol. Dr. Bernabe Bloj, Tucuman, 4000, Argent.). Analytical Biochemistry, 252(1), 186-189 (English) 1997. CODEN: ANBCA2. ISSN: 0003-2697. Publisher: Academic.

AB Using com. available thiosubstrates, such as 2,3-dimercapto-1-propanol tributyrate, the regiospecificities of 1,3-specific and nonspecific lipases was confirmed. The spectrophotometric test is a simple, rapid, and convenient alternative method to those previously reported for the characterization of the positional specificities of new lipases.

L10 ANSWER 15 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN
1997:621945 Document No. 127:302903 Human liver carboxylesterase hCE-1: binding specificity for cocaine, heroin, and their metabolites and analogs. Brzezinski, Monica R.; Spink, Benjamin J.; Dean, Robert A.; Berkman, Clifford E.; Cashman, John R.; Bosron, William F. (Departments of

Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN, 46202-5122, USA). Drug Metabolism and Disposition, 25(9), 1089-1096 (English) 1997. CODEN: DMDSAI. ISSN: 0090-9556. Publisher: Williams & Wilkins.

AB Purified human liver carboxylesterase (hCE-1) catalyzes the hydrolysis of cocaine to form benzoylecgonine, the deacetylation of heroin to form 6-acetylmorphine, and the ethanol-dependent transesterification of cocaine to form cocaethylene. In this study, the binding affinities of cocaine, cocaine metabolites and analogs, heroin, morphine, and 6-acetylmorphine for hCE-1 were evaluated by measuring their kinetic inhibition consts. with 4-methylumbelliferyl acetate in a rapid spectrophotometric assay. The naturally occurring (R)-(-)-cocaine isomer displayed the highest affinity of all cocaine and heroin analogs or metabolites. The pseudo- or allopseudococaine isomers of cocaine exhibited lower affinity indicating that binding to the enzyme is stereoselective. The Me ester, benzoyl, and N-Me groups of cocaine play important roles in binding because removal of these groups increased Ki values substantially. Compds. containing a variety of hydrophobic substitutions at the benzoyl group of cocaine bound to the enzyme with high affinity. The high Ki value obtained for cocaethylene relative to cocaine is consistent with weaker binding to the esterase and a longer elimination half-life reported for the metabolite. The spectrophotometric competitive inhibition assay used here represents an effective method to identify drug or environmental esters metabolized by carboxylesterases like hCE-1.

7/11/97

L10 ANSWER 16 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN

1997:417240 Document No. 127:132514 Quick E. A Fast Spectrophotometric Method To Measure the Enantioselectivity of Hydrolases. Janes, Lana E.; Kazlauskas, Romas J. (Department of Chemistry, McGill University, Montreal, QC, H3A 2K6, Can.). Journal of Organic Chemistry, 62(14), 4560-4561 (English) 1997. CODEN: JOCEAH. ISSN: 0022-3263. Publisher: American Chemical Society.

AB The slow step in finding and optimizing enantioselective biocatalysts is measuring their enantioselectivity. The current endpoint method for measuring enantioselectivity typically requires several hours to run a reaction, work it up, and measure the enantiomeric purity of the product and/or remaining starting material. The authors report a fast spectrophotometric method that measures enantioselectivity of hydrolases in less than one minute. The initial rates of hydrolysis of pure enantiomers of a chromogenic substrate, such as 4-nitrophenyl 2-phenylpropanoate, 1, are easily measured by monitoring the release of 4-nitrophenoxide at 404 nm. However, the ratio of sep. measured initial rates does not give the correct enantioselectivity because it ignores the competitive binding of the two enantiomers. To reintroduce competition, the authors added a chromogenic reference compound, resorufin tetradecanoate, to each reaction. In the first reaction, one enantiomer competes with the reference compound, while in the second reaction the other enantiomer competes with the same reference compound The ratio of the two relative rates gives the enantioselectivity. The quick E method agrees with the slower endpoint method for both low and high enantioselectivities and is fast enough to screen biocatalyst libraries.

L10 ANSWER 17 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN

1997:123499 Document No. 126:222088 Polythionate degradation by tetrathionate hydrolase of Thiobacillus ferrooxidans. De Jong, Govardus A. H.; Hazeu, Wim; Bos, Piet; Kuenen, J. Gijs (Department of Microbiology and Enzymology, Delft, University of Technology, Delft, 2628 BC, Neth.). Microbiology (Reading, United Kingdom), 143(2), 499-504 (English) 1997. CODEN: MROBEO. ISSN: 1350-0872. Publisher: Society for General Microbiology.

AB Cell-free exts. of Thiobacillus ferrooxidans grown with thiosulfate as energy source and prepared at high ammonium sulfate concns. and at low pH are capable of polythionate hydrolysis. The enzyme responsible for the hydrolysis of tetrathionate ($S_4O_6^{2-}$) and pentathionate ($S_5O_6^{2-}$) was purified to homogeneity. Enzyme activity during the purification procedure was based on a continuous

spectrophotometric method that detects soluble intermediates that absorb in the UV region. The end products of hydrolysis of both polythionates by the pure enzyme were thiosulfate, sulfur and sulfate. The purified enzyme has a pH optimum of around 4 and a temperature optimum of 65 °C. The activity is strongly influenced by the presence of sulfate ions. The purified enzyme is a dimer with two identical subunits of mol. mass 52 kDa. During purification of tetrathionate hydrolase, fractions able to hydrolyze trithionate and tetrathionate were separated, indicating that the two substrates are hydrolyzed by different enzymes.

4/19/96

L10 ANSWER 18 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN
1996:169520 Document No. 124:224777 Phosphoenolpyruvate Mutase Catalysis of Phosphoryl Transfer in Phosphoenolpyruvate: Kinetics and Mechanism of Phosphorus-Carbon Bond Formation. Kim, Jaebong; Dunaway-Mariano, Debra (Department of Chemistry and Biochemistry, University of Maryland, College Park, MD, 20742, USA). Biochemistry, 35(14), 4628-35 (English) 1996. CODEN: BICHAW. ISSN: 0006-2960. Publisher: American Chemical Society.

AB Phosphoenolpyruvate phosphomutase (PEP mutase) from Tetrahymena pyriformis catalyzes the rearrangement of phosphoenolpyruvate (PEP) to phosphonopyruvate (P-pyr). A spectrophotometric P-pyr assay consisting of the coupled actions of P-pyr decarboxylase, phosphonoacetaldehyde hydrolase, and alc. dehydrogenase was devised to monitor mutase catalysis. The reaction consts. determined for PEP mutase catalyzed conversion of PEP to P-pyr at pH 7.5 and 25 °C in the presence of Mg(II) are kcat = 5 s-1, Km = 0.77 ± 0.05 mM, and Keq = (2-9) + 10-4. In the PEP forming direction, kcat = 100 s-1 and Km = 3.5 ± 0.1 μM. Retention of stereochem. at phosphorus and strong inhibition displayed by the pyruvyl enolate analog, oxalate, have been cited as two lines of evidence that PEP mutase catalysis proceeds via a phosphoenzyme-pyruvyl enolate intermediate [Seidel, H. M., & Knowles, J. R. (1994) Biochem. 33, 5641-5646]. In this study, single turnover reactions of oxalyl phosphate with the PEP mutase were carried out to test the formation of the phosphoenzyme intermediate. If formed, the phosphoenzyme-oxalate complex should be sufficiently stable to isolate. Reaction of the mutase with [32P]oxalyl phosphate in the presence of Mg(II)/Mn(II) cofactor failed to produce a detectable level of the [32P]phosphoenzyme-oxalate complex. In contrast, the same reaction carried out with pyruvate phosphate dikinase (PPDK), an enzyme known to catalyze the phosphorylation of its active site histidine with PEP, occurred at a rate of 4 + 10-4 s-1 (15% E-P formed) in the presence Mg(II) and at a rate of 3 + 10-3 s-1 (60% E-P formed) in the presence of Mn(II). Both oxalyl phosphate (Ki = 180 ± 10 μM) and oxalate (Ki = 32 ± 10 μM) were competitive inhibitors of PEP mutase catalysis, but neither displayed slow, tight binding inhibition. These results do not support the intermediacy of a phosphoenzyme-pyruvyl enolate complex in PEP mutase catalysis.

L10 ANSWER 19 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN
1995:729417 Document No. 123:142292 Continuous spectrophotometric assay and properties of ascorbic acid oxidizing factors in wheat. Every, D.; Gilpin, M. J.; Larsen, N. G. (Grain Foods Research Unit, New Zealand Institute for Crop & Food Research, Christchurch, N. Z.). Journal of Cereal Science, 21(3), 231-9 (English) 1995. CODEN: JCSCDA. ISSN: 0733-5210. Publisher: Academic.

AB A continuous spectrophotometric assay was developed to measure ascorbic acid oxidation in crude Na2SO4 exts. of flour. The rate of ascorbic acid oxidation in flour exts. measured using this method was similar to the rate in flour-water suspensions and 2-4 fold less than the rate in dough measured using an indophenol-xylene extraction method. Flour exts. appeared to contain two ascorbic acid oxidizing factors; one with optimal activity at pH 6.3 and 30°C and the other with optimal activity at pH 10 and 40°C. The pH 6.3 factor had properties similar to those of ascorbate oxidase (EC 1.10.3.3) in its pH and temperature stability, strong inhibition by NaN3, KCN and diethyldithiocarbamate, inactivation by proteases, and greater stereospecificity towards L-ascorbic acid than D-isoascorbic acid. The pH 6.3 factor was most concentrated in the pollard

milling fraction of wheat and was lowest in flour. The pH 10 factor had several properties indicating non-enzymic oxidation of ascorbic acid; it was not inactivated by proteases, it was inhibited poorly or not at all by the above ascorbate oxidase inhibitors, and it had low specificity for stereoisomers of ascorbic acid.

L10 ANSWER 20 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN

1994:695765 Document No. 121:295765 Analysis of substrate cleavage by recombinant protease of human T cell leukemia virus type 1 reveals preferences and specificity of binding. Daenke, Susan; Schramm, Hans J.; Bangham, Charles R. M. (Mol. Immunol. Group, Inst. Mol. Med., Oxford, OX3 9DU, UK). Journal of General Virology, 75(9), 2233-9 (English) 1994. CODEN: JGVIAY. ISSN: 0022-1317.

AB Human T cell leukemia virus type 1 (HTLV-1) protease (PR14) was expressed in bacteria and purified by gel filtration. A continuous spectrophotometric assay was used to measure the kinetic parameters of substrate hydrolysis of PR14. Several peptide substrates containing HTLV-1 sequences known to be cleaved by PR14 were used. Cleavage anal. showed that the affinity with which PR14 binds these substrates is higher than that previously reported for HTLV-1 Gag peptides. Also, the affinities of peptides containing the sites involved in autocleavage of protease from its precursor are higher than for the peptides containing sites required for structural protein maturation. This suggests that the autocatalysis of protease from its own precursor has priority over other cleavage reactions and supports similar observations of an ordered hierarchy of processing events by retroviral proteases. As the N- and C-terminal regions of retroviral aspartic proteases are known to contribute to stability of the dimer by forming antiparallel β-strands, short peptides corresponding to these terminal sequences of HTLV-1 protease were tested for their ability to inhibit cleavage of substrates by PR14. Inhibition was seen with a C-terminal peptide corresponding exactly to the C-terminal 11 amino acids of the processed PR14, whereas a peptide containing a sequence situated further from the C terminus was less effective. An inhibitor of the protease of human immunodeficiency virus type 1, Ro 31-8959, was found to be a poor inhibitor of PR14.

L10 ANSWER 21 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN

1994:100171 Document No. 120:100171 Diastereomeric phosphonate ester adducts of chymotrypsin: 31P-NMR measurements. Kovach, Ildiko M.; McKay, Linda; Vander Velde, David (Dep. Chem., Cathol. Univ. America, Washington, DC, USA). Chirality, 5(3), 143-9 (English) 1993. CODEN: CHRLEP. ISSN: 0899-0042.

AB The generation of diastereomeric phosphonate ester adducts of chymotrypsin was evidenced for the 1st time by 31P NMR and spectrophotometric kinetic measurements. 31P NMR signals were recorded for 4-nitrophenyl-2-Pr methylphosphonate (IMN) at 32.2 ppm and for its hydrolysis product at 26.3 ppm downfield from phosphoric acid. The inhibition of chymotrypsin at pH >8.0 by the faster reacting enantiomer of IMN or 2-Pr methylphosphonochloridate (IMCl), or other phosphonate ester analogs of these compds., all caused a .apprx.6.0 ppm downfield shift of the 31P signal to the 39-40 ppm region. IMN, when applied below the stoichiometric amount of chymotrypsin, under the same conditions, generated 2 signals, at 39.0 and at 37.4 ppm. Scans accumulated in hourly intervals showed the decomposition of both diastereomers, with approx. half-lives of 12 h at pH 8.0 and 22°, into a species with a resonance at 35.5 ppm. The most likely reaction to account for the appearance of this new peak is the enzymic dealkylation of the iso-Pr group from the covalently bound phosphonate ester. The authors based this conclusion mostly on the similarity of the upfield shift to the hydrolysis of phosphonate esters. Contrary to experience with phosphate ester adducts of serine proteases, no signal was detected higher than 25.0 ppm downfield from phosphoric acid for several phosphonate ester adducts of chymotrypsin and in no case did the resonance for the adduct shift further downfield in the course of the expts.

L10 ANSWER 22 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN
1993:443970 Document No. 119:43970 The interaction of cytosolic epoxide hydrolase with chiral epoxides. Dietze, Eric C.; Kuwano, Eiichi; Hammock, Bruce D. (Dep. Entomol., Univ. California, Davis, CA, 95616, USA). International Journal of Biochemistry, 25(1), 43-52 (English) 1993. CODEN: IJBOBV. ISSN: 0020-711X.

AB The kinetic parameters of the cytosolic epoxide hydrolase were examined with two sets of spectrophotometric substrates. The (2S, 3S)- and (2R, 3R)-enantiomers of 4-nitrophenyl trans-2,3-epoxy-3- phenylpropyl carbonate had a KM of 33 and 68 μm and a Vmax of 16 and 27 μmol/min/mg, resp. With the (2S,3S)- and (2R,3R)-enantiomers of 4-nitrophenyl trans-2,3-epoxy-3-(4-nitrophenyl)propyl carbonate, cytosolic epoxide hydrolase had a KM of 8.0 and 15 μM and a Vmax of 7.8 and 5.0 μmol/min/mg, resp. Glycidyl 4-nitrobenzoate had the lowest I50 of the compds. tested in the glycidyl 4-nitrobenzoate series (I50 = 140 μM). The I50 of the (2R)-enantiomer was 3.7-fold higher. The inhibitor with the lowest I50 in the glycidol series, and the lowest I50 of any compound tested, was (2S,3S)-3-(4-nitrophenyl)glycidol (I50 = 13.0 μM). It also showed the greatest difference in I50 between the enantiomers (330-fold). All enantiomers of glycidyl 4-nitrobenzoates and trans-3-phenylglycidols gave differential inhibition of cytosolic epoxide hydrolase. However, neither the (S,S)-/(S)- or (R,R)-/(R)-enantiomer always had the lower I50. Addition of one or more Me groups to either enantiomer of glycidyl 4-nitrobenzoate resulted in increased I50. However, addition of a Me group to C2 of either enantiomer of 3-phenylglycidol resulted in a decreased I50. Finally, when the hydroxyl group of trans-3-(4-nitrophenyl)glycidol was esterified the I50 of the (2S,3S)- but not the (2R,3R)-enantiomer increased.

L10 ANSWER 23 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN
1993:191127 Document No. 118:191127 Synthesis of stereospecific esters and resolution of racemic alcohols with immobilized lipases . Sagiroglu, Ayten; Telefoncu, Azmi (Fac. Sci. Letters, Trakya Univ., Edirne, 22030, Turk.). Indian Journal of Chemistry, Section B: Organic Chemistry Including Medicinal Chemistry, 32B(1), 85-7 (English) 1993. CODEN: IJSBDB. ISSN: 0376-4699.

AB Immobilization of lipases from two different sources has been carried out by covalent bonding with agarose activated by CNBr. The product has been trapped in a polymeric gel matrix, absorbed on celite or macroporous Amberlite IRA-938 resin. Immobilized lipases prepared by using Amberlite IRA-938 are the most suitable biocatalysts for transesterification reactions. In these reactions, a matrix ester, racemic and nonracemic alcs. have been used as reactants. By using the immobilized lipases, the stereospecific esters have been synthesized. These esters give enantiomeric alcs. on hydrolysis in alkaline media. Thus, the resolution of racemic alcs., 2- and 3-pentanol, and 2-methyl-1-butanol, has been achieved. B.ps. and refractive indexes of all the products have been determined Sp. rotations of optically active products have also been recorded. Anal. of products has been done using chromatog. and spectrophotometric techniques (TLC, GLC and IR).

L10 ANSWER 24 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN
1992:628993 Document No. 117:228993 Mechanism of O-acetylserine sulfhydrylase from Salmonella typhimurium LT-2. Nalabolu, S. R.; Tai, C. H.; Schnackerz, K. D.; Cook, P. F. (Dep. Biochem. Mol. Biol., Texas Coll. Osteopath. Med., Fort Worth, TX, USA). Amino Acids, 2(1-2), 119-25 (English) 1992. CODEN: AACIE6. ISSN: 0939-4451.

AB O-Acetylserine sulfhydrylase (I) is a pyridoxal phosphate (PLP)-dependent enzyme that catalyzes the final step of L-cysteine biosynthesis in Salmonella, viz., the conversion of O-acetyl-L-serine (OAS) and sulfide to L-cysteine and acetate. A spectrophotometric assay was available using 5-thio(2-nitrobenzoate) (TNB) as an analog of sulfide and monitoring the disappearance of absorbance at 412 nm. I

catalyzed a ping pong mechanism with α-aminoacrylate in Schiff base with the active site PLP as a covalent intermediate. Using data obtained from the pH dependence of kinetic parameters, the acid-base chemical mechanism, and the optimum protonation state of the enzyme and substrate functional groups necessary for binding were determined The Schiff base and the α-amine of the substrate, OAS, were unprotonated for binding. There also appeared to be a requirement for 1 active-site general base to accept a proton from the α-amine and to donate a proton to form cysteine. I also catalyzed an OAS hydrolase activity, and the pH dependence of this reaction suggested that the active site lysine that participated in the Schiff base linkage is protonated to start the 2nd half-reaction, and has a pK of .apprx.8.2. The stereochem. of [3H]borohydride reduction of the Schiff base in free enzyme was determined by degradation of the resulting pyridoxyllysine to pyridoxamine and measuring 3H release with apoaspartate aminotransferase. The sequence around the active site lysine was determined as Asn-Pro-Ser-Phe-Ser-Val-Lys-Cys-Arg.

L10 ANSWER 25 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN

1992:422445 Document No. 117:22445 An unusual active site identified in a family of zinc metalloendopeptidases. Becker, Andrew B.; Roth, Richard A. (Sch. Med., Stanford Univ., Stanford, CA, 94305, USA). Proceedings of the National Academy of Sciences of the United States of America, 89(9), 3835-9 (English) 1992. CODEN: PNASA6. ISSN: 0027-8424.

AB An unusual active site has been identified in a family of zinc metalloendopeptidases that includes bacterial protease III and the human and Drosophila insulin-degrading enzymes. All of these enzymes have been characterized as metalloendopeptidases and purified protease III has been shown to contain stoichiometric levels of zinc. However, all three proteases lack the consensus sequence (HEXXH) described in the active site of other zinc metalloendopeptidases. Instead, these proteinases contain an inversion of this motif, HXXEH. To determine whether this region could represent the active site in these proteins, the two histidines in protease III were individually mutated to arginine and the glutamate was mutated to glutamine. All three mutants were devoid of proteolytic activity toward an exogenous substrate, insulin, as compared to the wild-type protease. Three lines of evidence indicate that this loss of activity in the mutants is not due to distortion of the three-dimensional structure of the protein: (i) the mutants are secreted into the periplasmic space and chromatograph normally; (ii) all three mutants are expressed at levels nearly identical to wild-type protein and do not appear to have an increased susceptibility to proteolysis in the bacteria; and (iii) the mutants compete equally with wild-type protein in a RIA. The purified wild-type and glutamate mutants were found to contain stoichiometric amts. of zinc by atomic absorption spectrophotometry , whereas both histidine mutants had negligible zinc signals. These findings are consistent with this region being the active site in this protein, with the histidine residues coordinating the essential zinc atom and the glutamate involved in catalysis.

L10 ANSWER 26 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN

1992:230665 Document No. 116:230665 Effect of salt on the kinetic parameters of retroviral and mammalian aspartic acid proteases. Tropea, Joseph E.; Nashed, Nashaat T.; Louis, John M.; Sayer, Jane M.; Jerina, Donald M. (Lab. Bioorg. Chem., Natl. Inst. Diabetes Dig. Kidney Dis., Bethesda, MD, 20892, USA). Bioorganic Chemistry, 20(1), 67-76 (English) 1992. CODEN: BOCMBM. ISSN: 0045-2068.

AB A continuous spectrophotometric method was used to measure the rates of hydrolysis of 3 synthetic peptide substrates by the retroviral proteases of human immunodeficiency virus type 1 (HIV-1) and of avian myeloblastosis virus (AMV), as well as the mammalian aspartic protease, pepsin. The kinetic parameter, kcat/Km, for these reactions was markedly increased by increasing the NaCl concentration In contrast to earlier reports, no evidence for a bell-shaped dependence of the rate on NaCl concentration was observed up to the highest salt concentration (5.0M) used. Detailed kinetic anal. of the hydrolysis of the synthetic peptide,

Thr-Phe-Gln-Ala-Phe(NO2)-Pro-Leu-Arg-Glu-Ala, catalyzed by AMV protease and by pepsin at NaCl concns. of 2-5M showed that the kcat remained constant, whereas the Km for both enzymes and the Ki for the inhibition by pepstatin A of the AMV protease-catalyzed hydrolysis decreased by .apprx.65-fold under these conditions. The observation of similar NaCl effects on kcat/Km for the homodimeric retroviral proteases and the monomeric enzyme, pepsin, was not consistent with a salt effect on the monomer-dimer equilibrium of the retroviral proteases. Furthermore, the lack of a salt effect on kcat also suggested that increasing the concentration of NaCl does not significantly alter the secondary structure nor the extent of dimerization of the retroviral proteases. The NaCl effect on Km and Ki was discussed in terms of a mechanism in which hydrophobic interactions between the inhibitor or substrate and the enzyme are enhanced by a salting out effect on the substrate and/or the active site region of the enzyme.

L10 ANSWER 27 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN

1991:37766 Document No. 114:37766 Resistance mechanisms in two color forms of the tobacco aphid (Homoptera: Aphididae). Harlow, C. D.; Lampert, E. P. (Dep. Entomol., North Carolina State Univ., Raleigh, NC, 27695, USA). Journal of Economic Entomology, 83(6), 2130-5 (English) 1990. CODEN: JEENAI. ISSN: 0022-0493.

AB Cultures of the tobacco aphid (Myzus nicotianae) were collected from tobacco throughout major tobacco-growing regions of North Carolina and maintained in culture in the laboratory Nine cultures of tobacco aphids were established. Four of these cultures were green in color; five were red. Slide-dip tests were performed with various insecticides and a known synergist. In tests with malathion, monocrotophos, acephate, and the esterase inhibitor S,S,S-tri-Bu phosphorotrithioate (DEF), resistance of aphids from the five red cultures and one green culture (from Duplin County) increased 3-4 times compared with the remaining three green cultures. In tests with endosulfan and methamidophos, a breakdown product of acephate, all nine cultures responded equally to treatment. Total carboxylesterase activities of aphids from all nine cultures were measured in a spectrophotometric assay. Aphids from the five red cultures and the green culture from Duplin County demonstrated ≈2.5 times the carboxylesterase activity as aphids from the remaining three green cultures. In all cultures, synergism of malathion, acephate, and methamidophos by DEF was apparent.

L10 ANSWER 28 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN

1989:610951 Document No. 111:210951 Inhibitory properties and antigenic specificity of monoclonal antibodies to pancreatic colipase. De la Fourniere, Laurence; Bosc-Bierne, Isabelle; Bellon, Bernard; Sarda, Louis (Lab. Biochim., Univ. Provence, Marseille, 13331, Fr.). Biochimica et Biophysica Acta, 998(2), 158-66 (English) 1989. CODEN: BBACAQ. ISSN: 0006-3002.

AB An immunochem. approach was used to understand the mechanism by which colipase (I) acts as a protein cofactor for anchoring pancreatic lipase at triacylglycerol/water interface. Ten monoclonal antibodies (Mabs) against porcine pancreatic pro-I were produced. Purified Igs and Fab fragments were studied for their capacity to inhibit I-dependent lipase activity. These studies were carried out by using pro-I, the secretory form of the cofactor, and its trypsin-treated form obtained by removal of the N-terminal pentapeptide by trypsin. The reactivities of Mabs with both forms of I were also studied by immunoenzymic methods. Mabs 6.1, 49.20, 75.8, 270.13, and 419.1 were found to inhibit lipolysis by preventing the binding of pro-I or trypsin-treated I to the lipid substrate. Mab 72.11 inhibited pro-I binding but had no effect on trypsin-treated I. Mab 72.11 reacted with pro-I in an ELISA, but showed no reactivity with trypsin-treated I. Finally, preincubation of Mab 72.11 with porcine pro-I prevented specific cleavage at the Arg-5-Gly-6 bond by trypsin. It was concluded, that the 1st 5 residues of pro-I are structural elements of the antigenic determinant recognized by Mab 72.11. The results of ELISA additivity tests (cotitrns.) further indicated that epitopes for Mabs 6.1, 72.11, 270.13 and 419.1 and for Mabs 49.20 and 75.8 were located in 2 distinct antigenic regions of

the pro-I mol. Apparently, the lipid-binding domain of pancreatic I comprises 2 regions. The 1st region corresponds to the N-terminal fragment of the protein. The 2nd region is likely identical with the peptide segment at position 51-59 as previously hypothesized from NMR and spectrophotometric studies. Studies carried out on pro-I chemical modified at tyrosine residues provided evidence that epitopes for Mabs 49.20 and 75.8 are in or close to the region which contains tyrosines at positions 55 and 59, and that the 2 peptide regions essential for interfacial binding are spatially adjacent in pro-I and the trypsin-treated form of I. General conclusions were in accordance with the location of antigenic regions of pro-I determined by predictive methods.

L10 ANSWER 29 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN
1988:33585 Document No. 108:33585 Malathion toxicity and carboxylesterase activity in Drosophila melanogaster. Ashour, Mohamed Bassem A.; Harshman, Lawrence G.; Hammock, Bruce D. (Dep. Entomol., Univ. California, Davis, CA, 95616, USA). Pesticide Biochemistry and Physiology, 29(2), 97-111 (English) 1987. CODEN: PCBPBS. ISSN: 0048-3575.

AB Adults from 9 strains of D. melanogaster were exposed to a residual film of malathion (I). The LC50 values indicated that there was a 4-fold difference in susceptibility to I among the strains. 3-Nonylthio-1,1,1- trifluoropropan-2-one (NTFP), a carboxylesterase inhibitor, synergized the toxicity of the insecticide when 4 fly strains were exposed to a mixture of the inhibitor and I. Whole-body homogenates of adults were analyzed by wide-range isoelec.-focusing (IEF). Carboxylesterase activities on the gels were stained using common substrates such as α- and β-naphthyl acetate and p-nitrophenyl acetate. Less conventional substrates, Et and iso-Bu carbonates of α-naphthol and p-nitrophenol, were synthesized and used for staining gels and kinetics in solution Two major bands of activity (pI 4.4 and 4.0) were detected with naphthyl substrates. A major peak of I hydrolytic activity (pI 6.3) was detected by running spectrophotometric assays for carboxylesterase activity on I using IEF gel slices and a rapid, semiautomated assay method. The peak region of activity on I did not coincide with bands obtained when naphthyl substrates were used to stain the gels. NTFP inhibited I hydrolysis in a solution from the IEF peak of activity and also inhibited esterase activity on α-naphthyl acetate on the gels. Recovered carboxylesterase activities on I and O-Et carbonate of p-nitrophenol were higher in the cytosolic than in the microsomal or mitochondrial cell fractions.

L10 ANSWER 30 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN
1985:609792 Document No. 103:209792 Biomimetic models for cysteine proteases. 3. Acylation of imidazolium-thiolate zwitterions by p-nitrophenylacetate as a model for the acylation step and demonstration of intramolecular general-base-catalyzed delivery of water by imidazole to thiol esters as a model for the deacylation step. Street, J. P.; Skorey, K. I.; Brown, R. S.; Ball, R. G. (Dep. Chem., Univ. Alberta, Edmonton, AB, T6G 2G2, Can.). Journal of the American Chemical Society, 107(25), 7669-79 (English) 1985. CODEN: JACSAT. ISSN: 0002-7863.

AB As biomimetic models for cysteine proteases, 4 imidazole-thiol pairs, 4(5)-(mercaptomethyl)imidazole, 2-(mercaptomethyl)imidazole, 2-(mercaptomethyl)-N-methylimidazole, and 2-(4,5-dimethylimidazol-2- yl)benzenethiol were studied (as a function of pH) as to their propensity to attack p-nitrophenyl acetate and dinitrophenyl acetate (for the latter pair). All species (except the 1st) attack through their thiolate forms and show a plateau region at intermediate pH values which is attributable to attack by the thiolate anion of the zwitterionic forms (ImH-S-). The 1st compound attacks as its thiolate at high pH and through imidazole N at neutrality. Potentiometric and UV-visible spectrophotometric titrns. establish quant. the microscopic pKa values from which are derived the fraction of individual species at any pH. General-base assistance of thiol attack on the acylating agent by the proximal imidazole is not required to explain the result. Deacylation of the corresponding thiol esters is studied as a function of pH, and in all cases, a plateau region from pH 6.5 to 8.5-10 is observed Solvent deuterium isotope effects from 1.88 to 3:75 are observed at

neutral pH values. In all cases, the origin of the plateau region stems from a general-base-promoted delivery of H2O to the thiol ester by the proximal imidazole. Trapping expts. with Ellman's reagent suggest that S → N-acyl transfer is not an important process for these systems. The relevance of these findings is discussed in terms of the mechanism of action of the cysteine proteases.

L10 ANSWER 31 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN
1980:464637 Document No. 93:64637 Hepatic microsomal epoxide hydrolase. Mechanistic studies of the hydration of K-region arene oxides. Armstrong, Richard Neil; Levin, Wayne; Jerina, Donald M. (Lab. Bioorg. Chem., Natl. Inst. Arthritis, Metab. Dig. Dis., Bethesda, MD, 20205, USA). Journal of Biological Chemistry, 255(10), 4698-705 (English) 1980. CODEN: JBCHA3. ISSN: 0021-9258.

AB The kinetic properties of microsomal and purified epoxide hydrolase toward K-region arene oxide substrates have been investigated by a direct spectrophotometric technique. The kinetic mechanism of the membrane-bound and purified enzymes was studied with phenanthrene 9,10-oxide (I) as substrate. The catalytic rate constant (kc) and Km for the hydration of the substrate were 0.55 s-1 and 1.1 μM, resp., for the microsomal enzyme and 0.61 s-1 and 1.8 μM, resp, for the purified enzyme. The temperature dependence of kc for both enzymes was similar, suggesting that the active sites of the 2 enzymes are indistinguishable with respect to the kinetics and thermodn. of hydration of I. The pH dependence of kc indicated that the decomposition of the enzyme substrate complex to products is dependent on an ionization with an apparent pKα of 6.7. Furthermore, a kinetic solvent isotope effect of kc(H2O)/kc(D2O) = 1.52 and a neg. entropy of activation for kc were found. These data are consistent with a mechanism involving general base catalysis of the nucleophilic addition of water to epoxides by a histidine residue at the active site. Nonionic detergents, which were necessary to solubilize highly lipophilic substrates, inhibit the action of epoxide by hydrolase. The relatively water-soluble substrate, I, was used to probe the mechanism of the inhibition. Both detailed kinetic studies and direct binding expts. indicated that a simple mechanism involving substrate sequestration by detergent micelles was sufficient to explain the inhibition. Epoxide hydrolase was found to exhibit a marked enantiomeric selectivity toward the chiral K-region arene oxides, benzo[a]pyrene 4,5-oxide, benzo[a]anthracene 5,6-oxide, and 3-bromophenanthrene 9,10-oxide, in detergent solution The 40-fold difference in the rate of hydration of (+)- and (-)-benzo[a]pyrene 4,5-oxide may be of particular importance in the biol. activity of this highly mutagenic K-region arene oxide.

L10 ANSWER 32 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN
1980:210711 Document No. 92:210711 Spectrophotometric determination of the proteolytic activity of enzyme preparations of microbiological origin in the UV and visible regions. Fonberg-Broczek, Monika; Urbanek-Karlowska, Bogumila (Pol.). Metody Badania Prep. Enzym., 11-16. Panstw. Zakl. Hig.: Warsaw, Pol. (Polish) 1977. CODEN: 43DIAC.

AB Denatured Hb was incubated at 35.5° for 30 min with bacterial or fungal protease at pH 7.0 or 4.7, resp. Trichloroacetic acid at 5% was added to stop hydrolysis and dissolve its products which were determined in the filtrate or centrifugation supernatant by absorption at 275 nm. Alternatively, the sample was treated with 3.5N NaOH and dilute Folin-Ciocalteu reagent, and the resulting blue color was determined by absorption at 660 nm.

L10 ANSWER 33 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN
1977:596240 Document No. 87:196240 Substrate specificity of human α-L-fucosidase. Dawson, Glyn; Tsay, Grace (Pritzker Sch. Med., Univ. Chicago, Chicago, IL, USA). Archives of Biochemistry and

Biophysics, 184(1), 12-23 (English) 1977. CODEN: ABBIA4. ISSN: 0003-9861.

AB Human α-L-fucosidase is shown to be a soluble lysosomal enzyme which hydrolyzes α-L-fucose residues linked to the 2-position of galactose or the 3, 4, or 6 position of N-acetylglucosamine. Demonstration of activity of human α-L-fucosidase towards natural oligosaccharide or glycosphingolipid substrates was achieved by measuring liberated L-fucose by coupling to fucose dehydrogenase and NAD and measuring NADH production spectrophotometrically. Activity of purified human spleen, brain, and cultured skin fibroblast or crude cell exts. towards 4-methylumbelliferyl-α-L-fucoside had a pH optimum of 4.5-5.5 and was unaffected by the presence of neutral detergents such as Triton X-100. However, the addition of Na taurocholate or other bile salts to the incubation mixture caused a marked inhibition of pH 5 and a shift in pH optimum to the pH 6-7 region. Na taurocholate effected a 3-fold reduction in the apparent Km for α-L-fucosidase at pH 6.0, but studies on fucosidosis tissue (α-fucosidase deficiency) or subcellular fractions derived from rat liver failed to indicate the existence of a membrane-bound α-L-fucosidase. The response of other lysosomal hydrolases to the presence of bile salts was investigated and was found to be variable, perhaps depending upon the hydrophilic or hydrophobic nature of the natural substrate and (or) the state of association of the active enzyme.

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1976:28129 Document No. 84:28129 Effects of senescence on the protein complement of plasma membranes from cotyledons. Lees, G. L.; Thompson, J. E. (Dep. Biol., Univ. Waterloo, Waterloo, ON, Can.). New Phytologist, 75(3), 525-32 (English) 1975. CODEN: NEPHAV. ISSN: 0028-646X.

AB The protein composition of plasma membranes from young and senescent cotyledons of germinating Phaseolus vulgaris were analyzed by polyacrylamide gel electrophoresis after solubilizing the membranes in Na dodecyl sulfate. Membranes from 2-day-old tissue, an age still largely unaffected by senescence, showed 18 bands ranging in mol. weight from 12,000 to 150,000. Of these, 9 were sufficiently intense to register in spectrophotometric scans. Two fast-running proteins of mol. wts. 12,000 and 26,000 were quant. preponderant at this stage and comprised 11 and 25% of the total membrane protein, resp. With the onset of senescence during late germination the protein banding pattern changed. The sharp, clearly defined bands characteristic of gels from young membranes, were replaced by broad diffuse regions of protein stain in the gels from senescent membranes. In addition, the number of protein bands seen in the gels decreased between days 2 and 9 of germination. These observations suggest that membrane senescence in cotyledons is mediated, at least in part, by cytosol protease acting on membrane proteins. Such action would lead to complete breakdown of some proteins and alteration of others.

L10 ANSWER 35 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN

1975:455253 Document No. 83:55253 State and reactivity of tryptophyl residues in two bacterial proteases from Sorangium species. Leskovac, Vladimir (Dep. Chem., Univ. Novi Sad, Novi Sad, Yugoslavia). Biochimica et Biophysica Acta, 393(2), 563-70 (English) 1975. CODEN: BBACAQ. ISSN: 0006-3002.

AB The state and reactivity of tryptophyll residues in 2 proteolytic enzymes from Sorangium were investigated by spectrophotometric oxidation of tryptophans with N-bromosuccinimide, 2-hydroxy-5- nitrobenzylbromide, and H2O2 in dioxane, ORD, UV difference spectrophotometry, solvent perturbation, and viscosity measurements. Out of 2 tryptophyl residues/mol. of α-lytic protease, 1 appeared to be completely buried, whereas the other seemed to be exposed. None of these 2 residues were responsible for the activity of the enzyme. The β-lytic protease underwent an irreversible conformational transition between pH 5.0 and 3.5. Out of total 4 tryptophyl residues/mol., only 1 was fully exposed at neutral pH. The other 3 were gradually exposed in the pH transition region. The degree of exposure and the dimensions of cavities shielding tryptophyl residues were

estimated The tryptophyl residues of β-lytic protease did not seem to participate in substrate binding or the active site; they were rather one of the determinants of the conformational state of the enzyme.

L10 ANSWER 36 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN
1974:45102 Document No. 80:45102 Nature of the serum enzyme catalyzing paraoxon hydrolysis. Lenz, David E.; Deguehery, Lindsey E.; Holton, James S. (Basic Med. Sci. Dep., Edgewood Arsenal, MD, USA). Biochimica et Biophysica Acta, 321(1), 189-96 (English) 1973. CODEN: BBACAQ. ISSN: 0006-3002.

AB A modified spectrophotometric method for analyzing the enzymic activity of the serum enzyme which catalyzes the hydrolysis of paraoxon (E-600) was developed. The reaction is not subject to either substrate or product inhibition. Neither p-aminophenyl diethyl phosphate nor p-aminophenyl pinacolyl methylphosphonate were substrates, but were competitive inhibitors with Ki values of 1.02 + 10-3M and 4.4 + 10-4M, respectively. The enzyme active site apparently contains a hydrophobic region at the binding site and requires an electron withdrawing group in the substrate to ensure that cleavage occurs through the P-O bond or to stabilize an anionic site at the active site.

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1974:1131 Document No. 80:1131 Fluorescence studies of the bacterial protease E-30-III. Jori, Giulio; Genov, Nikolai (Ist. Chim. Org., Univ. Padova, Padua, Italy). International Journal of Peptide & Protein Research, 5(3), 171-7 (English) 1973. CODEN: IJPPC3. ISSN: 0367-8377.

AB The fluorescence emission of the bacterial protease E-30-III, irradiated with 290-nm light, is exclusively due to the tryptophyl residues, which appear to be located in a rather polar environment. One indole side chain, which is fully exposed to the aqueous solvent, gives a minor contribution to the overall emission of the protein. On the other hand, the 2 strongly emitting indole systems appear to be contained in internal regions having a relatively high dielectric const; fluorescence quenching studies demonstrate that the efficiency of the fluorescence emission by the 2 residues is about the same. In particular, 1 tryptophyl side chain must be spatially adjacent to a glutamyl or aspartyl residue. Exposure of the protein to 280-nm and 290-nm radiation reveals the occurrence of an efficient energy transfer from tyrosine to tryptophan in the neutral pH region and from tryptophan to tyrosinate in alkaline solns; the latter process parallels the ionization of the phenolic side chains, as determined by spectrophotometric titrations. In acid solns., the electronic energy transfer among aromatic residues is greatly diminished, and clearly resolved emission spectra from the tyrosyl and tryptophyl residues are observed

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1969:76033 Document No. 70:76033 Relation of the hemolytic activity of active C'1s to its TAMe [p-tosyl-l-arginine methylester] esterase action: purification and assay. Nagaki, Kazuyoshi; Stroud, Robert M. (Birmingham Veterans Admin. Hosp., Birmingham, AL, USA). Journal of Immunology, 102(2), 421-30 (English) 1969. CODEN: JOIMA3. ISSN: 0022-1767.

AB Active C'1s (first component of human complement) was isolated from the euglobulin fraction of human serum by CM- and DEAE-cellulose column chromatog. and Pevikon block electrophoresis. The highly purified active C'1s showed on immunoelectrophoresis a single precipitin line against horse anti-whole human serum. The esterase activity of C'1s was measured spectrophotometrically using TAME as a substrate. The esterase and the hemolytic activities, as well as the C'2 and C'4 destructive activity, of active C'1s were associated with a single homogeneous protein (mol. weight .apprx.110,000) which migrated to the α-globulin region.

L10 ANSWER 39 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN

1967:514336 Document No. 67:114336 Oxidoreductive and hydrolytic enzyme patterns in plant suspension culture cells. Local and time relations. De Jong, Donald W.; Jansen, Eugene F.; Olson, Alfred C. (Western Regional Res. Lab., USDA, Albany, CA, USA). Experimental Cell Research, 47(1-2), 139-56 (English) 1967. CODEN: ECREAL. ISSN: 0014-4827.

AB Cytochem. tests were used to determine the patterns of enzyme activities in WR-132 suspension cultures. A geometrical periodicity was observed in 3-day-old cells for several dehydrogenases. The pattern was 3 neg. cells to 1 pos. cell for malate and succinate dehydrogenase, but the reverse for ethanol and 3-hydroxybutyrate dehydrogenase. Enzymes which did not demonstrate this periodicity in 3-day-old cells were acetylesterase, acid phosphatase, and cytochrome oxidase. On the basis of cytochem. localization, enzyme activity was assigned to specific intracellular sites: acetylesterase in endoplasmic reticulum, acid phosphatase in cell wall, glucose-6-phosphate dehydrogenase in spherosomes and lipid-rich particles, succinate dehydrogenase in plastid-like structures as well as in mitochondria, glutamate dehydrogenase in particles heavily concentrated within cytoplasmic regions adjacent to the crosswall. Spectrophotometric analyses of formazan exts. obtained following dehydrogenase reactions demonstrated that specific enzyme activity in the cell cultures oscillated over the 12-day growth period. Four activity profiles were obtained for the 6 dehydrogenases examined The data suggested a correlation between succinate and glutamate dehydrogenase and also ethanol and 3-hydroxybutyrate dehydrogenase. The maximum and min. in the activity curve for malate dehydrogenase were distinct. 23 references.

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1967:400254 Document No. 67:254 Serum cholinesterases--comparison of the spectrophotometric and Acholest test paper methods. Schmidinger, St.; Doenicke, Alfred (Univ. Munich, Munich, Germany). Zeitschrift fuer Klinische Chemie, 4(6), 273-81 (German) 1966. CODEN: ZKLCAY. ISSN: 0372-9184.

AB The spectrophotometric method of Kalow (K. and Lindsay, Can. J. Biochem. Physiol. 33: 568(1958)) and the Acholest test paper method for serum cholinesterase (I) were tested and evaluated in parallel detns. on 722 persons. The following results were obtained on the basis of statistical calcns.: for normal and for pathol. decreased levels of I, there was a constant function and a very good correlation between the 2 methods. The physiol. distribution of I by both methods averaged 23.5%; with decreasing enzymic activity, the Kalow method gave a lower average distribution. The reciprocal dependence of the 2 methods is destroyed by the presence of an atypical serum I. With an atypical I, however, the Acholest values tend more to the pathol. region than the Kalow values. Genetically determined atypical enzyme variation can thus be detected indirectly by Acholest. Exptl. details of the various methods are given.

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1967:198 Document No. 66:198 Thermostable protease from thermophilic bacteria. I. Thermostability, physicochemical properties, and amino acid composition. Ohta, Yumiko; Ogura, Yasuyuki; Wada, Akiyoshi (Univ. Tokyo, Tokyo, Japan). Journal of Biological Chemistry, 241(24), 5919-25 (English) 1966. CODEN: JBCHA3. ISSN: 0021-9258.

AB The effect of temperature on the enzyme activity of the protease produced by Bacillus thermoproteolyticus was examined in the temperature range between 25 and 88°. The kinetic analysis of the enzyme which was heated for 1 hr. at 80° indicates that the heated enzyme has the same maximum velocity but lower affinity for substrate than the native one. Mol. weight of the protease was found to be 37,500. The value of intrinsic viscosity was 3.3 ml./g. The ionization behavior of the tyrosine residue of the native and the heat-treated enzyme was examined by spectrophotometric titration. The titration curves indicated that nearly two-thirds of the tyrosine residues in the native enzyme ionized abnormally above pH 10.5, but there were no abnormally ionizing tyrosines in the heat-treated enzyme.

The analysis of the fluorescence spectra of tryptophan residues in the native enzyme was carried out in comparison with that of tryptophan in various solvents. These expts. suggest that some tryptophan residues of the enzyme in the native state are buried in a nonpolar environment with high refractive index rather than exposed to water. From the measurement of the optical rotatory dispersion in the 300- to 600-mμ spectral zone and the uv region, it appeared that there was a low helix content in the native enzyme. The Cotton effect caused by tyrosine residues was observed by the measurement of the rotatory dispersion and circular dichroism. Amino acid composition of the enzyme was determined by column chromatography with the use of the automatic amino acid analyzer. The structure of the thermostable protease was discussed on the basis of the physicochem. properties of the enzyme. 18 references.

L10 ANSWER 42 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN

1964:435255 Document No. 61:35255 Original Reference No. 61:6189h,6190a-d Oxidative enzymes and hydrolytic enzymes in spinal neurons. I. Histochemical and biochemical investigation of the spinal ganglion and the spinal cord following sciatic neurotomy in the guinea pig. Kumamoto, T.; Bourne, G. H. (Wakayama Med. Coll., Japan). Acta Anatomica, 55(3), 255-77 (English) 1963. CODEN: ACATA5. ISSN: 0001-5180.

AB The left sciatic nerves of 300-400-g. adult male guinea pigs were cut 5-7 mm. from the level of the greater trochanter. The animals were sacrificed at intervals of 4, 8, 12, 22, and 32 days after the neurotomy and 3 spinal ganglia from the lumbosacral region and the lumbosacral segment of the spinal cord at the origin of the sciatic nerve were removed rapidly and placed in dry ice chambers. Histol. sections were stained for cytochrome oxidase (I) (Moog, CA 38, 33352), diphosphopyridine nucleotide (DPN)-diaphorase (II) (Nachlas, et al., CA 52, 10270a), non-specific esterase (III) (Pearse, CA 48, 12852b), succinic dehydrogenase (IV) (N., et al., CA 52, 6469g) and Nissl substance. Lactic dehydrogenase (V) (Wroblewski and La Due, CA 50, 2700e) and IV were determined in homogenates of 6 spinal ganglia and 2 spinal cord segments. The determination of IV was based on the reaction of a 1 mg./ml. nitro-BT solution in a tissue homogenate-0.1M Na succinate system incubated 1 hr. at 37°. The dye was extracted with BuOH and measured spectrophotometrically at 510 mμ. The activity of I decreased slowly in spinal ganglion cells and motor neurons of the spinal cord up to 8 days after neurotomy and then markedly from 8 to 11 days after neurotomy, with no significant change thereafter. The activity of IV showed a strong to moderate pos. reaction in the gray matter of the spinal cord and a strong pos. reaction in the glial cells on the outer surface of motor neurons in normal and chromatolytic cells. The localization of IV activity changed in the chromatolytic cycle and in spinal ganglion cells on or about 8 days after the neurotomy. Maximum IV activity was seen in the central zone of the chromatolytic cells accompanied by reduced IV activity in the peripheral cytoplasm. Changes in II activity could not be well visualized during chromatolysis because of its mitochondrial and microsomal origin. A slight decrease in III activity was seen in both neurons and spinal ganglia. Assays of IV and V activity 8 days after neurotomy indicated a decrease in IV of 15.07 and 7.33% below normal in spinal ganglia and spinal cord, resp. and no significant change in V activity in the same tissues.

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1964:39888 Document No. 60:39888 Original Reference No. 60:7077f-h Sulfur metabolism of Aerobacter aerogenes. I. A repressible sulfatase. Rammler, D. H.; Grado, C.; Fowler, L. R. (Natl. Insts. of Health, Bethesda, MD). Biochemistry (Moscow, Russian Federation), 3(2), 224-30 (Unavailable) 1964. CODEN: BIORAK. ISSN: 0006-2979.

AB When A. aerogenes (American Type Culture Collection strain 9621) is grown on a synthetic medium containing a growth-limiting amount of sulfate or with methionine as its sole source of S, sulfatase synthesis occurs. This enzyme is repressed by sulfate, thiosulfate, sulfite, and cysteine in normal growth concns. It is readily assayed in whole cells, either in liquid media or on agar plates,

the assay consisting of spectrophotometrically following the liberation of p-nitrophenol from the enzymic hydrolysis of p-nitrophenyl sulfate. Derepression of the enzyme as a function of various S sources added to a sulfateless synthetic medium was studied. Methionine and its analogs provide the highest amts. of enzyme, while substances such as cystine or lanthionine are less effective in derepressing enzyme synthesis. Phosphate inhibits the enzymic hydrolysis of p-nitrophenyl sulfate, while sulfate has no effect. The optimum temperature for enzyme synthesis is in the region of 28°; higher or lower temps. retard enzyme formation. Chloramphenicol prevents enzyme formation. Although the exact biol. function of the enzyme is not known, it is assumed that it functions as an esterase, providing the cell with sulfate for growth. The ease of assay with whole cells and the inability to increase enzymic activity by rupturing the cells suggest that this is another of a class of enzymes bound to the surface of the cell.

L10 ANSWER 44 OF 44 CAPLUS COPYRIGHT 2005 ACS on STN

1926:13102 Document No. 20:13102 Original Reference No. 20:1632a-b Hematoporphyrin. I. Some optical properties of this pigment. Application to the measurement of hematoporphyrin in Harder's gland in the white rat. II. Study of the photosensibilizing action of hematoporphyrin on the red corpuscles. Fabre, R.; Simonnet, H. Bulletin de la Societe de Chimie Biologique, 8, 56-66 (Unavailable) 1926. CODEN: BSCIA3. ISSN: 0037-9042.

AB cf. Fabre, C. A., 20, 545, 722, and preceding abstract By using the spectrophotometric determination of the partition of intensity on the fluorescent spectrum, the quantity of hematoporphyrin in Harder's gland in the white rat is per pair of glands of mean weight 0.18 g. from 1.25 to 1.5 + 10-3 g. The photosensibilizing action appears to be exclusively produced by radiations from the yellow region of the spectrum.